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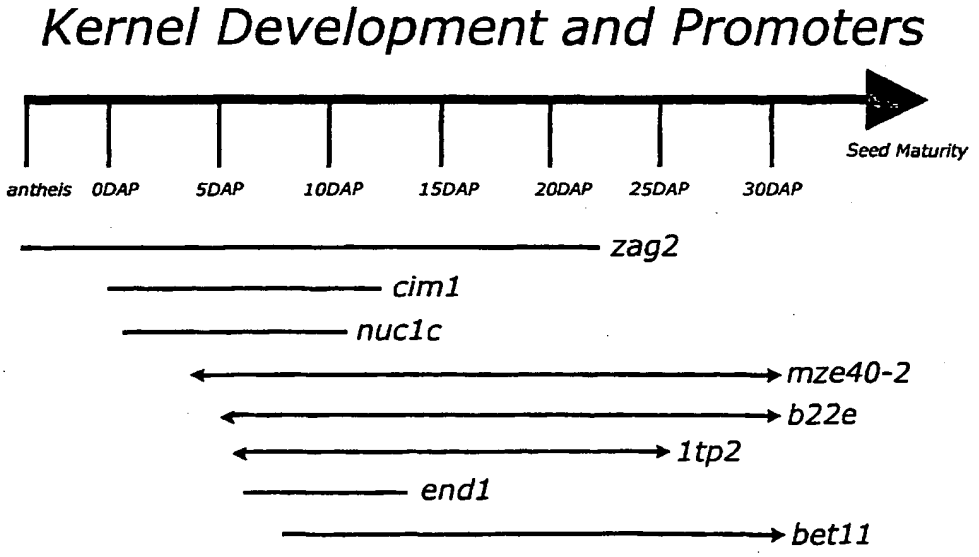
(54) Title: ENHANCED STRESS TOLERANCE IN MAIZE VIA MANIPULATION OF CELL CYCLE REGULATORY GENES



(57) Abstract: A transgenic method for enhancing cell division in female reproductive organs of plants is described. The genes are temporally and spatially expressed to affect the activation and/or modulation of cyclin-dependent kinases in a plant organ or tissue. Expression constructs and methods for the production of crop plants with heritable phenotypes which are useful for breeding programs designed to increase yield potential over a range of environmental conditions are also included.

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TITLE: ENHANCED STRESS TOLERANCE IN MAIZE VIA MANIPULATION OF CELL CYCLE REGULATORY GENES

FIELD OF THE INVENTION

This invention relates generally to the field of plant molecular biology. More specifically, this invention relates to methods and reagents for the temporal and spatial expression of genes that enhance cell division in plants, especially transgenic plants, to increase yield and health of crop plants in general as well as in periods of stress.

BACKGROUND OF THE INVENTION

Cell division plays a crucial role during all phases of plant development. The continuation of organogenesis and growth responses to a changing environment require precise spatial, temporal and developmental regulation of cell division activity in meristems (and in cells with the capability to form new meristems, such as in lateral root formation). Control of cell division is also important in organs themselves (i.e., separate from meristems *per se*), for example, in leaf expansion, secondary growth, and endoreduplication.

A complex network controls cell division in eukaryotes. Various regulatory pathways communicate environmental constraints such as nutrient availability, mitogenic signals such as growth factors or hormones, or developmental cues such as the transition from vegetative to reproductive growth. Ultimately, these regulatory pathways control the timing, rate, plane, and position of cell division.

Cell division in higher eukaryotes is controlled by two main checkpoints in the cell cycle which prevent the cell from entering either M- or S-phase prematurely. Evidence from yeast and mammalian systems has repeatedly shown that over-expression of key cell cycle genes can either trigger cell division in non-dividing cells, or stimulate division in previously dividing cells (i.e., the duration of the cell cycle is decreased and cell size is reduced). Examples of genes whose over-expression has been shown to stimulate cell division include cyclins (see, e.g., Doerner, P. *et al.*, Nature (1996) 380:520-523; Wang, T.C. *et al.*, Nature (1994) 369:669-671; Quelle, D.E. *et al.*, Genes Dev. (1993) 7:1559-1571); E2F

transcription factors (see, e.g., Johnson, D.G. *et al.*, Nature (1993) 365:349-352; Lukas, J. *et al.*, Mol. Cell. Biol. (1996) 16:1047-1057) , cdc25 (see, e.g., Bell, M.H. *et al.*, Plant Mol. Bio. (1993) 23:445-451; Draetta, D. *et al.*, BBA (1996) 1332:53-63), and mdm2 (see, e.g., Teoh, G. *et al.*, Blood (1997) 90:1982-1992). Conversely, other gene products have been found to participate in checkpoint control, effectively blocking or retarding progression through the cell cycle (Cebolla *et al.*, EMBO 18(16):4476-84(1999)).

The basic mechanism of cell cycle control is conserved among eukaryotes. A catalytic protein kinase and an activating cyclin subunit control progress through the cell cycle. The protein kinase is generally referred to as a cyclin-dependent-kinase (CDK), whose activity is modulated by phosphorylation and dephosphorylation events and by association with regulatory subunits called cyclins. CDKs require association with cyclins for activation, and the timing of activation is largely dependent upon cyclin expression.

Eukaryote genomes typically encode multiple cyclin and CDK genes. In higher eukaryotes, different members of the CDK family act in different stages of the cell cycle. Cyclin genes are classified according to the timing of their appearance during the cell cycle. In addition to cyclin and CDK subunits, CDKs are often physically associated with other proteins which alter localization, substrate specificity, or activity. A few examples of such CDK interacting proteins are the CDK inhibitors, members of the Retinoblastoma-associated protein (Rb) family, and the Constitutive Kinase Subunit (CKS).

The protein kinase activity of the complex is regulated by feedback control at certain checkpoints. At such checkpoints the CDK activity becomes limiting for further progress. When the feedback control network senses the completion of a checkpoint, CDK is activated and the cell passes through to the next checkpoint. Changes in CDK activity are regulated at multiple levels, including reversible phosphorylation of the cell cycle factors, changes in subcellular localization of the complex, and the rates of synthesis and destruction of limiting components. Regulation of the cell cycle by the cyclin/CDK complex is noted particularly at the G1/S phase transition and at the G2/M phase transition. P.W. Doerner, *Cell Cycle Regulation in Plants*, Plant Physiol. (1994) 106:823-827.

Plants have unique developmental features that distinguish them from other eukaryotes. Plant cells do not migrate, and thus only cell division, expansion, and programmed cell death determine morphogenesis. Organs are formed throughout the entire life span of the plant from specialized regions called meristems. In addition, many differentiated cells have the potential both to dedifferentiate and to reenter the cell cycle. There are also numerous examples of plant cell types that undergo endoreduplication, a process involving nuclear multiplication without cytokinesis. The study of plant cell cycle control genes is expected to contribute to the understanding of these unique phenomena. O. Shaul *et al.*, *Regulation of Cell Division in Arabidopsis*, Critical Reviews in Plant Sciences, 15(2):97-112 (1996).

Current methods for genetic engineering in maize require a specific cell type as the recipient of new DNA. These cells are found in relatively undifferentiated, rapidly growing callus cells or on the scutellar surface of the immature embryo (which gives rise to callus). There is evidence to suggest that cells must be dividing for transformation to occur. Therefore, to optimize transformation it would be desirable to provide a method for increasing the number of cells undergoing division.

It has also been observed that dividing cells represent only a fraction of cells that transiently express a transgene. Regardless of the delivery method currently used, DNA is introduced into literally thousands of cells, yet transformants are recovered at frequencies of 10^{-5} relative to transiently-expressing cells. The presence of damaged DNA in non-plant systems (similar to DNA introduced by particle gun or other physical means) has been well documented to rapidly induce cell cycle arrest. Siede, W., *Cell cycle arrest in response to DNA damage: lessons from yeast*. Mutation Res. 337(2):73-84 (1995). An increase in understanding and control of the cell cycle could also help to further increase the rate of recovery of transformants.

Anthesis is generally recognized as the critical period of ear and kernel development in maize. Varied experimental approaches demonstrate that treatments, which decrease the cell division around anthesis, decrease grain yield. For example, large yield losses occur when maize plants are subjected to

abscisic acid (ABA) (Myers, P.N. *et al.*, 1990; Mambelli and Setter, 1998), thermal stress (Jones, R.J. *et al.*, 1985; Cheikh and Jones, 1994), water-deficits (Artlip, T.S. *et al.*, 1995) or exposed to high plant density around anthesis. (*See* Zinselmeier, C. and J.E. Habben, Use of mRNA-Profiling Technology to Determine Gene Expression Patterns in Developing Maize Ears that Differ in Yield, Plant Physiology Abstracts (1998); Prine, G.M. *A Critical Period for Ear Development in Maize*, Crop Science 11:782-786 (1971).) Conversely, treatments that increase plant cell division around anthesis increase grain yield. For example, application of cytokinins (Lejeune, P. *et al.*, 1998). In most cases, the variation in yield was related to the number of kernels that developed. Collectively, these results suggest that kernel number and size may be limited by cell division, particularly during drought or high density stress at anthesis. According to the invention, enhancing cell division of the immature ear and grain would maintain ear and seed growth, and as a consequence, buffer this important vulnerable period of yield formation.

The tissues targeted for transgenes are in the maize female inflorescence, since relative to other organs, it is frequently the most sensitive to abiotic stress. For example, transient water stress prior to pollination has been shown to arrest the growth of ears, embryo sacs, and silks. After pollination, drought stress can inhibit endosperm cell division, which peaks at 8 to 10 days after pollination. As a result, both kernel set and endosperm development are inhibited. This effect is most pronounced in the apical region of the ear. Retarded endosperm development can result in aborted apical kernels, because of reduced cell division and decreased endoreduplication. Not surprisingly, both of these events have been shown to be controlled by cyclin dependent protein kinases.

Barrennesss (the lack of ear development) is one of the most common manifestations of maize plants grown at high densities. Another prevalent trait in density stressed plants is an increase in the anthesis/silking interval, which has been shown to be the result of retarded ear growth. Based on this and other information, one key to producing a viable ear under plant-population stress is to maintain its growth rate. Since cell division is a key component of organ growth,

the cell cycle regulatory mechanism in the female inflorescence is the target for expression of transgenes.

Traditional methods of improving yield formation have centered around breeding techniques. As with any valuable plant species, breeders have long used conventional breeding techniques to improve yield. While improvements have been achieved, breeding techniques are laborious and slow because of the time required to breed and grow successive plant generations. Furthermore, certain phenotypes may be impossible to obtain by conventional techniques. Thus, it would be desirable to utilize recombinant DNA technology to produce new plant varieties and cultivars in a controlled and predictable manner. It would be especially desirable to produce crop and ornamental plants with improved seed set over a range of environmental conditions to increase yield potential.

It can be seen from the foregoing that a need exists in the art for a transgenic method of increasing yield potential in plants.

It is an object of the present invention to provide expression constructs which when expressed in a temporal and spatial manner in a transgenic plant increase yield potential, as well as resistance to stress through regulation of cell division.

It is another object of this invention to provide transgenic plant lines with heritable phenotypes which are useful in breeding programs designed to increase yield potential in crop plants over a range of environmental conditions.

It is yet another object of this invention to produce seed which will produce plants with increased yield potential.

It is a further object of this invention to provide plants, plant cells, and plant tissues containing the expression constructs of the invention.

Other objects of the invention will become apparent from the description of the invention which follow.

SUMMARY OF THE INVENTION

The present invention comprises the spatial and temporal expression of a nucleotide sequence which will enhance stress tolerance (buffer female inflorescence), particularly high density and drought stresses, in plants at critical

times in plant development such as the vulnerable time of anthesis. In particular, this invention relates to polynucleotides which encode proteins involved in the regulation of the cell cycle. More particularly, the polynucleotides encode proteins which enhance cell division in maize ears and kernels by directly increasing the activities of cyclin dependent protein kinases or indirectly by augmenting the activity of enzymes which control CDK activity.

Cell division in higher eukaryotes is controlled by a well-conserved mechanism. The principal control factor of this mechanism is a protein threonine/serine kinase complex that is composed of cyclin (the regulatory subunit) and CDK (the catalytic subunit). This complex controls cell division by phosphorylating target proteins. Eukaryotes have evolved an elaborate regulatory network to safeguard the fluctuation of CDK activities in the cell cycle. Cyclins oscillate in abundance as a result of both transcriptional and post-transcriptional regulation. This provides an on/off control for CDK, since the association of cyclin is absolutely required for kinase activity. Phosphorylation and dephosphorylation of CDK occurs. Three important phosphorylation sites are involved in modulating CDK activities. Phosphorylation of Tyr161 by the CDK activating kinase (CAK) activates CDK, while phosphorylation of Thr14 and Tyr15 by Myt1 and Wee1, respectively, inactivates CDK (Mueller, P.R. *et al.*, Mol. Biol. Cell 6, 119(1995); Mueller, P.R. *et al.*, Science 270, 86 (1995)). CDC25, a protein tyrosine phosphatase dephosphorylates Tyr15 and activates CDK (Kumagai, A. and Dunphy, W.G., Cell 70, 139 (1992). Both Wee1 and CDC25 are in turn regulated by phosphorylation. Nim1, a protein kinase identified in *S. pombe* is able to phosphorylate Wee1 (this inhibits Wee1 activity), while P1x1 is able to use CDC25 as a substrate and enhance CDC25 activity, a positive feedback loop for CDK regulation. The CDK complex interacts with CDK inhibitors (CKIs). A number of proteins can physically bind to CDK and inhibit CDK activity. Well-characterized inhibitors in human systems include p21, p27, p57, p16, and p19.

Identification of rate-limiting pathways influenced by abiotic stresses are important in determining which ones to target. Carbohydrate and nitrogen metabolic pathways, as well as hormonal pathways, have been found to be modulated by stress. A recent study of wheat (Schuppler, U. *et al.*, "Effect of

Water Stress on Cell Division and Cell-Division-Cycle 2-Like Cell-Cycle Kinase Activity in Wheat Leaves," Plant Physiol. 117: 667-678 (1998)) showed convincing evidence that proteins encoded by cell cycle genes can be targets of water stress. When a transient drought was imposed on the wheat seedlings, the mesophyll cells of leaves were arrested at the G1 phase. Enzyme assays revealed that there was a 50% decrease in CDK activity in the cells, which was caused by an increased level of Tyr15 phosphorylation.

Apical kernel abortion is a common characteristic of maize subjected to drought stress. Research has shown that the plant hormone cytokinin, is able to reduce apical kernel abortion. Concurrently, it was shown that cytokinin can enhance CDK activities by reducing the extent of CDK phosphorylation at Tyr15. Other research has shown that the cell cycle regulatory mechanism is highly conserved among all eukaryotes. Cell cycle genes from maize, *Arabidopsis*, and alfalfa are able to rescue yeast mutants that are defective in cell cycle genes. Likewise, yeast cell cycle genes, such as CDC25, are able to promote cell division in higher plants. Thus, heterologous genes will work in transgenic maize events.

In one embodiment, the invention comprises a genetic construct which upon expression in plant cells provides a DNA sequence encoding a gene product useful for directing the phosphorylation or activation state of CDK of a plant or plant tissue. Particularly, B-type and D-type cyclins, CDC25, Nim1, and P1x1 will be over expressed in order to promote cell division under stress. In another embodiment, the invention comprises a genetic construct which provides a DNA sequence encoding a gene product useful for co-suppressing Wee1 in order to promote cell division of a plant or plant tissue.

Kernel abortion increases when unfavorable environments occur around flowering, thereby decreasing genetic yield potential in plants. Typically, developing female florets are more prone to abiotic stress compared to male florets. CDKs are critical enzymes that determine maize floral cell division. Modification of female cell division by altering the activation of CDKs in a tissue and temporal specific manner should increase the likelihood of vigorous female floral development and also improve the consistency of seed set under unfavorable conditions.

Thus the invention contemplates expression of cell division enhancing nucleotide sequences during vulnerable periods, primarily those involved with anthesis development, where yield is most significantly affected by stress.

Definitions

As used herein the term "anthesis development" shall include any period in plant development where yield may be more significantly impacted by stress. This can include the exponential growth phase of the ear during which biomass is accumulated and the lag phase of kernel development as more fully described herein and in the following references. ("Set and Flower Synchrony within the Ear of Maize II. Plant Population Effects", Crop Science, 37: 448-455 (March-April 1997); and Shaw, Robert "Climate Requirement", Corn Improvement, 3rd ed., Chapter 10, pp. 609-638). As shown in Figure 1, reprinted from Corn and Corn Improvement, plant yields are most vulnerable to moisture stress at a time period centered around flowering (0-10 DAP). Typically, this period will be approximately 14 days prior to flowering through approximately 14 days after flowering.

The examples and discussion herein may specifically reference maize, however the teachings herein are equally applicable to any other grain or flowering crop.

As used herein the term "ear" shall not be limited to maize and shall include any developing female inflorescence from a plant.

As used herein the term "kernel" shall also not be limited to maize but shall include grain, or seed within a fruit.

As used herein the term "cell division enhancing nucleotide sequence" shall mean any nucleotide sequence, (DNA, RNA, coding and/or antisense) the expression of which increases the rate of a particular plant tissue's cell division as compared to the rate without the expression of said sequence.

According to the invention, a genetic construct is disclosed which causes expression of the cell division enhancing nucleotide sequence at a time and location to maximize cell division typically during very vulnerable periods primarily, around anthesis. The spatial and temporal expression of genes

affecting cell division of tissues can be achieved using different types of promoters. Promoters useful for the invention are promoters which would cause the temporal and spatial expression of a gene product during anthesis as defined herein and can be constitutive, inducible, or tissue specific.

For example, seed specific promoters can be used to enhance cell division during seed development, pre-pollination promoters can also be used or stress inducible promoters can be used to enhance cell division during periods of stress. The optimization of promoters to achieve the objectives of the invention is considered routine and easily ascertainable by those of skill in the art and is intended to be within the scope of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic diagram (reproduced from Shaw, Robert "Climate Requirement", Corn Improvement, 3rd ed., Chapter 10, pp. 609-638). As shown in Figure 1, reprinted from Corn and Corn Improvement from p. 614) of the relationship between age of crop and percentage yield decrement due to 1 day of moisture stress. The top and bottom lines represent the highest and lowest yield reductions obtained in stress experiments, the middle line the average reduction.

Figure 2 is a chart depicting expression timing of various promoters useful for the present invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

The present invention is based on isolation and characterization of genes affecting CDKs or enzymes which control CDKs which control cell division in plants. Any nucleotide sequence encoding an enzyme in the CDK activation/modulation (phosphorylation/dephosphorylation) pathways may be used in accordance with the present invention. Nucleotide sequences encoding these enzymes are easily ascertainable to those of skill in the art through Genbank or the references disclosed herein. Other reactions and pathways may be utilized by different organs in a plant or by different plant species. By changing the levels or activity of a component in the

activation/deactivation/modulation pathway, it is possible to affect the levels of cell division in the plant, plant organ, or plant tissue.

Many different types of CDKs have been identified in plants. Several cDNAs encoding functional homologs of cdc2 kinase have been isolated by reduced stringency hybridization or reverse transcription coupled polymerase chain reaction from a number of plant species, including pea (Feiler and Jacobs, 1990), alfalfa (Hirt *et al.*, 1991, 1993), Arabidopsis (Ferreira *et al.*, 1991; Hirayama *et al.*, 1991), soybean (Miao *et al.*, 1993), Antirrhinum (Fobert *et al.*, 1994), and maize (Colasanti *et al.*, 1991).). Soni, R. *et al.*, "A Family of Cyclin D Homologs from Plants Differentially Controlled by Growth Regulators and Containing the Conserved Retinoblastoma Protein Interaction Motif", The Plant Cell, 7:86 (1995). Several other CDKs have been cloned and are easily accessible to those of skill in the art.

At least three different types of cyclins have been identified in plants: A-type homologs, B-type homologs, and D-type homologs (Renaudin, J-P *et al.*, "Plant cyclins: a unified nomenclature for plant A-, B- and D-type cyclins based on sequence organization", Plant Mol. Biol., 32:1003-1018 (1996)). A-type cyclins are broken down into three structural groups (A1, A2, and A3). Cyclin A1 has been isolated from maize. (Renaudin *et al.*, Table 1). B-type cyclins are broken down into two structural groups (B1, and B2). Cyclins B1 and B2 have been isolated from maize. (Renaudin *et al.*, Table 1). D-type cyclins contain three structural groups (D1, D2, and D3). A number of cDNA sequences encoding plant mitotic cyclins with A- or B-type characteristics of having mixed A- and B-type features have been isolated from various species, including carrot (Hata *et al.*, 1991), soybean (Hata *et al.*, 1991), Arabidopsis (Hermerly *et al.*, 1992; Day and Reddy, 1994), alfalfa (Hirt *et al.*, 1992), Antirrhinum (Fobert *et al.*, 1994) and maize (Redaudin *et al.*, 1994; Sun, Y. *et al.*, 1997, CycZmIn from maize endosperm (GenBank #U66607), CycZme1, GenBank #U66608). Soni, R. *et al.*, "A Family of Cyclin D Homologs from Plants Differentially Controlled by Growth Regulators and Containing the Conserved Retinoblastoma Protein Interaction Motif", The Plant Cell, 7:86 (1995). Several other cyclins have been cloned and are easily accessible to those of skill in the art.

At its simplest, the invention comprises a nucleotide construct comprising a cell division enhancing nucleotide sequence, a regulatory promoter to regulate temporal tissue and spatial expression during anthesis development and termination sequences operably linked to said cell division enhancing sequence.

A non-exclusive list of enzymes that might be candidates for such intervention include Myt1, Wee1, Nim1, CDC25, P1x1, CKIs, CAK, and cyclins.

Identification of other polynucleotides which may be useful in the invention will typically be based on screening for procaryotic or eucaryotic organisms with altered levels of cell division using assays standard in the art and described herein. For example, and not limited to, plant hormones such as cyktokinins, ABA (Myers, P.N. *et al.* 1990), and auxin (Trehin *et al.*, planta (1998) 206(2):215-224)

The polynucleotides useful in the invention can be formed from a variety of different polynucleotides (e.g., genomic or cDNA, RNA, synthetic oligonucleotides, and polynucleotides), as well as by a variety of different techniques. As used herein, a polynucleotide is a sequence of either eukaryotic or prokaryotic synthetic invention.

In a preferred embodiment, the invention comprises use of one or more nucleotide sequences which, when expressed together enhance reproductive cell division. This can allow for hybrid plant or seed production, once transgenic inbred parental lines have been established. For this embodiment, the invention comprises a DNA sequence encoding B- or D-type cyclins, CDC25, Nim1, and/or P1x1 capable of promoting cell division by activating or modulating the activity of CDKs in critical, stress sensitive periods of plant development. In a second embodiment, DNA sequence encoding for suppression of Wee1 capable of promoting cell division by modulating the activity of CDKS, is provided for increasing yield, seed development, flowering or resistance to stress.

The invention is not limited to any plant type and can be used for any crop or ornamental plant species for which it is desirable to increase yield. The methods of the invention may be applicable to any species of seed-bearing plant to enhance yield potential by affecting the cell division in seed tissue.

The nucleotide constructs of the present invention will share similar elements, which are well known in the art of plant molecular biology. For example, in each construct the DNA sequences of interest will preferably be operably linked (i.e., positioned to ensure the functioning of) to a promoter which allows the DNA to be transcribed (into an RNA transcript) and will comprise a vector which includes a replication system. In preferred embodiments, the DNA sequence of interest will be of exogenous origin in an effort to prevent co-suppression of the endogenous genes.

Promoters (and other regulatory elements) may be heterologous (i.e., not naturally operably linked to a DNA sequence from the same organism). Promoters useful for expression in plants are known in the art and can be inducible, constitutive, tissue-specific, derived from eukaryotes, prokaryotes, or viruses, or have various combinations of these characteristics.

In choosing a promoter to use in the methods of the invention, it may be desirable to use a tissue-specific or developmentally regulated promoter. A tissue-specific or developmentally regulated promoter is a DNA sequence which regulates the expression of a DNA sequence selectively in the cells/tissues of a plant critical to seed set and/or function and/or limits the expression of such a DNA sequence to the period of seed maturation in the plant. Any identifiable promoter may be used in the methods of the present invention which causes expression during anthesis development as defined herein. It may also be advantageous to use a stress inducible promoter to provide expression of the construct during periods of stress.

Differential screening techniques can be used to isolate promoters expressed in developing female reproductive organs (kernels and/or immature ears) from around 14 days before pollination to approximately 12 days after pollination. Promoters predicted to operate in this manner include LTP2, gamma-zein, and ZAG2.

Promoters preferred for the invention would be acceptably timed to 14 days before and 12 days after anthesis when both immature ear and mitotically active kernel are most susceptible to the stress. Promoters predicted to operate during these developmental stages include LTP2, MZE40, nuc1 and ZAG2. For example,

LTP2 promoter from Barley (Kalla *et al.*, 1994, Plant J.6(6):849-860) confers the specificity of aleurone expression. Pioneer Researchers have shown that this promoter is also functional in maize. When fused with a GUS reporter gene, LTP2 promoter directed aleurone specific expression of GUS activity in maize kernels (Niu and Tome, unpublished). Aleurone is a single celled, out-most layer of endosperm that retains mitotic activity when the central region of endosperm ceased division and committed to endoreduplication. Therefore, LTP2 promoter will allow us to manipulate endosperm cell division when fused with cell division regulatory genes. B22E: 69 NAL Call No. 442.8 Z34 "Primary Structure of a Novel Barley Gene Differentially Expressed in Immature Alleurone Layers," Klemsdae, S.S. *et al.*, Springer Int'l 1991 Aug., Molecular and General Genetics, Vol. 228(1/2) p. 9-16, 1991. Expression of B22E is specific to the pedicel in developing maize kernels, Zag2: 134 NAL Call. No.: QK725.P532 Identification and molecular characterization of ZAG1, the maize homolog of the Arabidopsis floral homeotic gene AGAMOUS. Schmidt, R.J.; Veit, B.; Mandel, M.A.; Mena, M.; Hake, S.; Yanofsky, M.F. Rockville, MD: American Society of Plant Physiologists, c1989-; 1993 Jul. The Plant Cell v. 5(7): p 729-737; 1993 Jul. includes references. Zag2 transcripts can be detected 5 days prior to pollination to 7 to 8 DAP, and directs expression in the carpel of developing female inflorescences and Cim1 which is specific to the nucellus of developing maize kernels. Cim1 transcript is detected 4 to 5 days before pollination to 6 to 8 DAP. Other useful promoters include any promoter which can be derived from a gene whose expression is maternally associated with developing female florets.

Table 1 shows a list of preferred promoters including their timing of expression (DAP = days after pollination).

Promoter Expression Summary

Promoter	Source	Primary Tissue	Temporal
ltp2	barley cDNA	aleurone	<6 – 24+ DAP
cim1	maize EST	pericarp (under silk scar)	0 – 12+ DAP

mze40-2	maize EST	gloom, pericarp, pedicel forming region, low in scutellum	<4 – 28+ DAP
b22e	barley genomic	aleurone, embryo scutellum, pedicel forming region	<5 – 30+ DAP
zag2	maize, EST	floret, ovule	<0 – 22 DAP
endl	maize, cDNA	endosperm transfer cells	6 – 14 DAP
betl1	maize, cDNA	endosperm transfer cells	8 – 30+ DAP

Figure 2 also depicts the timing of various preferred promoters and kernel development.

For example a construct useful for the present invention might include the maize B-cyclin gene operably linked to the ZAG2 promoter for expression of B-cyclin <0 to 22 days after pollination.

Other promoters which are seed or embryo specific and may be useful in the invention include patatin (potato tubers) (Rocha-Sosa, M. *et al.* (1989) EMBO J. 8:23-29), convicilin, vicilin, and legumin (pea cotyledons) (Rerie, W.G., *et al.* (1991) Mol. Gen. Genet. 259:149-157; Newbigin, E.J., *et al.* (1990) Planta 180:461-470; Higgins, T.J.V., *et al.* (1988) Plant. Mol. Biol. 11:683-695), zein (maize endosperm) (Schemthaner, J.P., *et al.* (1988) EMBO J. 7:1249-1255), phaseolin (bean cotyledon) (Segupta-Gopalan, C. *et al.* (1985) Proc. Natl. Acad. Sci. U.S.A. 82:3320-3324), phytohemagglutinin (bean cotyledon) (Voelker, T. *et al.* (1987) EMBO J. 6:3571-3577), B-conglycinin and glycinin (soybean cotyledon) (Chen, Z-L *et al.* (1988) EMBO J. 7:297-302), glutelin (rice endosperm), hordein (barley endosperm) (Marris, C. *et al.* (1988) Plant Mol. Biol. 10:359-366), glutenin and gliadin (wheat endosperm) (Colot, V. *et al.* (1987) EMBO J. 6:3559-3564), and sporamin (sweet potato tuberous root) (Hattori, T. *et al.* (1990) Plant Mol. Biol. 14:595-604). Promoters of seed-specific genes operably linked to heterologous coding regions in chimeric gene constructions maintain their temporal and spatial expression pattern in transgenic plants. Such examples include *Arabidopsis thaliana* 2S seed storage protein gene promoter to express enkephalin peptides in

Arabidopsis and *Brassica napus* seeds (Vanderkerckhove *et al.*, Bio/Technology 7:L929-932 (1989)), been lectin and bean β-phaseolin promoters to express luciferase (Riggs *et al.*, Plant Sci. 63:47-57 (1989)), and wheat glutenin promoters to express chloramphenicol acetyl transferase (Colot *et al.*, EMBO J 6:3559-3564 (1987)).

Any inducible promoter can be used in the instant invention to temporarily express a particular construct during reproductive development. *See* Ward *et al. Plant Mol. Biol.*22: 361-366 (1993). Exemplary inducible promoters include, but are not limited to, that from the ACEl system which responds to copper (Mett *et al. PNAS* 90: 4567-4571 (1993)); In2 gene from maize which responds to benzenesulfonamide herbicide safeners (Hershey *et al., Mol. Gen. Genetics* 227: 229-237 (1991) and Gatz *et al., Mol. Gen. Genetics* 243: 32-38 (1994)) or Tet repressor from Tn10 (Gatz *et al., Mol. Gen. Genet.* 227: 229-237 (1991). A particularly preferred inducible promoter is a promoter that responds to an inducing agent to which plants do not normally respond. An exemplary inducible promoter is the inducible promoter from a steroid hormone gene, the transcriptional activity of which is induced by a glucocorticosteroid hormone. Schena *et al., Proc. Natl. Acad. Sci. U.S.A.* 88: 0421 (1991).

Many different constitutive promoters can also potentially be utilized in the instant invention. Exemplary constitutive promoters include, but are not limited to, the promoters from plant viruses such as the 35S promoter from CaMV (Odell *et al., Nature* 313: 810-812 (1985) and the promoters from such genes as rice actin (McElroy *et al., Plant Cell* 2: 163-171 (1990)); ubiquitin (Christensen *et al., Plant Mol. Biol* 12: 619-632 (1989) and Christensen *et al., Plant Mol. Biol.* 18: 675-689 (1992)): pEMU (Last *et al., Theor. Appl. Genet.* 81: 581-588 (1991)); MAS (Velten *et al., EMBO J.* 3: 2723-2730 (1984)) and maize H3 histone (Lepetit *et al., Mol. Gen. Genet.* 231: 276-285 (1992) and Atanassova *et al., Plant Journal* 2 (3): 291-300 (1992)).

The ALS promoter, a Xbal/*Nco*l fragment 5' to the *Brassica napus* ALS3 structural gene (or a nucleotide sequence that has substantial sequence similarity to said *Xbal/Nco*l fragment), represents a particularly useful constitutive promoter. See PCT application WO96/30530.

Transport of protein produced by transgenes to a subcellular compartment such as the nucleus, chloroplast, vacuole, peroxisome, glyoxysome, cell wall or mitochondrion, or for secretion into the apoplast, is accomplished by means of operably linking the nucleotide sequence encoding a signal sequence to the 5' and/or 3' region of a gene encoding the protein of interest. Targeting sequences at the 5' and/or 3' end of the structural gene may determine, during protein synthesis and processing, where the encoded protein is ultimately compartmentalized. The presence of a signal sequence directs a polypeptide to either an intracellular organelle or subcellular compartment or for secretion to the apoplast. Many signal sequences are known in the art. See, for example, Sullivan, T., "Analysis of Maize *Brittle*-1 Alleles and a Defective Suppressor-Mutator-Induced Mutable Allele", The Plant Cell, 3:1337-1348 (1991), Becker *et al., Plant Mol. Biol.*20: 49 (1992), Close, P.S., Master's Thesis, Iowa State University (1993), Knox, C., *et al.*, "Structure and Organization of Two Divergent Alpha-Amylase Genes From Barley", *Plant Mol.Biol.* 9: 3-17 (1987), Lerner *et al., Plant Physiol.*91: 124-129 (1989), Fontes *et al.,Plant Cell* 3: 483-496 (1991), Matsuoka *et al., Proc. Natl. Acad. Sci.* 88: 834 (1991), Gould *et al., J. Cell Biol* 108: 1657 (1989), Creissen *et al., Plant J.* 2: 129 (1991), Kalderon, D., Robers, B., Richardson, W., and Smith A., "A short amino acid sequence able to specify nuclear location", *Cell* 39: 499-509 (1984), Stiefel, V., Ruiz-Avila, L., Raz R., Valles M., Gomez J., Pages M., Martinez-Izquierdo J., Ludevid M., Landale J., Nelson T., and Puigdomenech P., "Expression of a maize cell wall hydroxyproline-rich glycoprotein gene in early leaf and root vascular differentiation", *Plant Cell* 2: 785-793 (1990).

Selection of an appropriate vector is relatively simple, as the constraints are minimal. The minimal traits of the vector are that the desired nucleic acid sequence be introduced in a relatively intact state. Thus, any vector which will produce a plant carrying the introduced DNA sequence should be sufficient. Typically, an expression vector contains (1) prokaryotic DNA elements encoding for a bacterial replication origin and an antibiotic resistance marker to provide for the growth and selection of the expression vector in a bacterial host; (2) DNA elements that control initiation of transcription, such as a promoter; (3) DNA elements that control the processing of transcripts such as transcription

termination/polyadenylation sequences; and (4) a reporter gene. Useful reporter genes include β-glucuronidase, β-galactosidase, chloramphynical acetyltransferase, luciferase, kanamycin or the herbicide resistance genes PAT and BAR. Preferably, the selectable marker gene is kanamyacin or the herbicide resistance genes PAT and BAR. The BAR or PAT gene is used with the selecting agent Bialaphos, and is used as a preferred selection marker gene for plant transformation (Spencer, *et al.* (1990) J. Thero. Appl'd Genetics 79:625-631). (5) The target or structural gene of interest.

One commonly used selectable marker gene for plant transformation is the neomycin phosphotransferase II *(nptll)* gene, isolated from transposon Tn5, which when placed under the control of plant regulatory signals confers resistance to kanamycin. Fraley *et al., Proc. Natl. Acad. Sci. U.S.A.,* 80: 4803 (1983). Another commonly used selectable marker gene is the hygromycin phosphotransferase gene which confers resistance to the antibiotic hygromycin. Vanden Elzen *et al., Plant Mol. Biol.,* 5: 299 (1985).

Additional selectable marker genes of bacterial origin that confer resistance to antibiotics include gentamycin acetyl transferase, streptomycin phosphotransferase, aminoglycoside- 3′ -adenyl transferase, the bleomycin resistance determinant. Hayford *et al., Plant Physiol.* 86: 1216 (1988), Jones *et al., Mol. Gen. Genet.,* 210: 86 (1987), Svab *et al., Plant Mol.. Biol..* 14: 197 (1990), Hille *et al., Plant Mol. Biol.* 7: 171 (1986). Other selectable marker genes confer resistance to herbicides such as glyphosate, glufosinate or broxynil. Comai *et al., Nature* 317: 741-744 (1985), Gordon-Kamm *et al., Plant Cell* 2: 603-618 (1990) and Stalker *et al., Science* 242: 419-423 (1988).

Other selectable marker genes for plant transformation are not of bacterial origin. These genes include, for example, mouse dihydrofolate reductase, plant 5 - *enol*pyruvylshikimate-3 -phosphate synthase and plant acetolactate synthase. Eichholtz *et al., Somatic Cell Mol. Genet.* 13: 67 (1987), Shah *et al., Science* 233: 478 (1986), Charest *et al., Plant Cell Rep.* 8: 643 (1990).

Another class of marker genes for plant transformation require screening of presumptively transformed plant cells rather than direct genetic selection of transformed cells for resistance to a toxic substance such as an antibiotic. These

genes are particularly useful to quantify or visualize the spatial pattern of expression of a gene in specific tissues and are frequently referred to as reporter genes because they can be fused to a gene or gene regulatory sequence for the investigation of gene expression. Commonly used genes for screening presumptively transformed cells include β-glucuronidase *(GUS)*, β-galactosidase, luciferase and chloramphenicol acetyltransferase. Jefferson, R.A., *Plant Mol. Biol. Rep.* 5*:* 387 (1987)., Teeri *et al., EMBO J.* 8: 343 (1989), Koncz *et al., Proc. Natl. Acad. Sci. U.S.A.* 84:131 (1987), De Block *et al., EMBO J.* 3: 1681 (1984). Another approach to the identification of relatively rare transformation events has been use of a gene that encodes a dominant constitutive regulator of the *Zea mays* anthocyanin pigmentation pathway. Ludwig *et al., Science* 247: 449 (1990).

Recently, *in vivo* methods for visualizing GUS activity that do not require destruction of plant tissue have been made available. Molecular Probes Publication 2908, Imagene Green, p. 1-4 (1993) and Naleway *et al., J. Cell Biol.*115: 15la (1991). However, these *in vivo* methods for visualizing GUS activity have not proven useful for recovery of transformed cells because of low sensitivity, high fluorescent backgrounds, and limitations associated with the use of luciferase genes as selectable markers.

More recently, a gene encoding Green Fluorescent Protein (GFP) has been utilized as a marker for gene expression in prokaryotic and eukaryotic cells. Chalfie *et al., Science* 263: 802 (1994). GFP and mutants of GFP may be used as screenable markers.

Genes included in expression vectors must be driven by a nucleotide sequence comprising a regulatory element, for example, a promoter. Several types of promoters are now well-known in the transformation arts, as are other regulatory elements that can be used alone or in combination with promoters.

A general description of plant expression vectors and reporter genes can be found in Gruber, *et al.* (Gruber *et al.* (1993) Vectors for Plant Transformation. In: Methods in Plant Molecular Biology and Biotechnology. Glich *et al.*, eds. (CRC Press), pp. 89-119.)

Expression vectors containing genomic or synthetic fragments can be introduced into protoplast or into intact tissues or isolated cells. Preferably

expression vectors are introduced into intact tissue. General methods of culturing plant tissues are provided, for example, by Maki, *et al.* (Maki, *et al.* (1993) Procedures for Introducing Foreign DNA into Plants: In: Methods in Plant Molecular Biology & Biotechnology; Glich *et al.* eds. (CRC Press), pp. 67-88; Philips, *et al.* (1988) Cell-Tissue Culture and In Vitro Manipulation. In Corn & Corn Improvement, 3rd ed. Sprague, *et al.* eds. (American Society of Agronomy Inc.), pp. 345-387).

Methods of introducing expression vectors into plant tissue include the direct transfection or co-cultivation of plant cell with *Agrobacterium tumefaciens* (Horsch *et al.* (1985) Science, 227:1229). Descriptions of *Agrobacterium* vector systems and methods for *Agrobacterium*-mediated gene transfer are provided by Gruber *et al.* (supra).

Numerous methods for plant transformation have been developed, including biological and physical, plant transformation protocols. See, for example, Miki *et al.*, "Procedures for Introducing Foreign DNA into Plants" in *Methods in Plant Molecular Biology and Biotechnology*, Glick, B.R. and Thompson, J.E. Eds. (CRC Press, Inc., Boca Raton, 1993) pages 67-88. In addition, expression vectors and *in vitro* culture methods for plant cell or tissue transformation and regeneration of plants are available. See, for example, Gruber *et al.*, "Vectors for Plant Transformation" in *Methods in Plant Molecular Biology and Biotechnology*, Glick, B.R. and Thompson, J.E. Eds. (CRC Press, Inc., Boca Raton, 1993) pages 89-119.

A. *Agrobacterium*-mediated Transformation

One method for introducing an expression vector into plants is based on the natural transformation system of *Agrobacterium*. See, for example, Horsch *et al., Science* 227: 1229 (1985). *A. tumefaciens* and *A. rhizogenes* are plant pathogenic soil bacteria which genetically transform plant cells. The Ti and Ri plasmids of *A. tumefaciens* and *A. rhizogenes*, respectively, carry genes responsible for genetic transformation of the plant. See, for example, Kado, C.I., *Crit. Rev. Plant. Sci.* 10: 1 (1991). Descriptions of *Agrobacterium* vector systems and methods for *Agrobacterium*-mediated gene transfer are provided by Gruber *et al.*,

supra, Miki *et al.*, *supra*, and Moloney *et al.*, *Plant Cell Reports* 8: 238 (1989). See also, U.S. Patent No. 5,591,616, issued Jan. 7, 1997.

B. Direct Gene Transfer

Despite the fact the host range for *Agrobacterium*-mediated transformation is broad, some major cereal crop species and gymnosperms have generally been recalcitrant to this mode of gene transfer, even though some success has recently been achieved in rice and maize. Hiei *et al.*, *The Plant Journal* 6: 271-282 (1994); U.S. Patent No. 5,591,616, issued Jan. 7, 1997. Several methods of plant transformation, collectively referred to as direct gene transfer, have been developed as an alternative to *Agrobacterium*-mediated transformation.

A generally applicable method of plant transformation is microprojectile-mediated transformation wherein DNA is carried on the surface of microprojectiles measuring 1 to 4 mm. The expression vector is introduced into plant tissues with a biolistic device that accelerates the microprojectiles to speeds of 300 to 600 m/s which is sufficient to penetrate plant cell walls and membranes. Sanford *et al.*, *Part. Sci. Technol.* 5: 27 (1987), Sanford, J.C., *Trends Biotech.* 6: 299 (1988), Klein *et al.*, *Bio/Technology* 6: 559-563 (1988), Sanford, J.C., *Physiol Plant* 79: 206 (1990), Klein *et al.*, *Biotechnology* 10: 268 (1992). In maize, several target tissues can be bombarded with DNA-coated microprojectiles in order to produce transgenic plants, including, for example, callus (Type I or Type II), immature embryos, and meristematic tissue.

Another method for physical delivery of DNA to plants is sonication of target cells. Zhang *et al.*, *Bio/Technology* 9: 996 (1991). Alternatively, liposome or spheroplast fusion have been used to introduce expression vectors into plants. Deshayes *et al.*, *EMBO J.*, 4: 2731 (1985), Christou *et al.*, *Proc Natl. Acad. Sci. U.S.A.* 84: 3962 (1987). Direct uptake of DNA into protoplasts using $CaCl_2$ precipitation, polyvinyl alcohol, or poly-L-ornithine have also been reported. Hain *et al.*, *Mol. Gen. Genet.* 199: 161 (1985) and Draper *et al.*, *Plant Cell Physiol.* 23: 451 (1982). Electroporation of protoplasts and whole cells and tissues have also been described. Donn *et al.*, In Abstracts of VIIth International Congress on Plant Cell and Tissue Culture IAPTC, A2-38, p 53 (1990); D'Halluin *et al.*, *Plant Cell* 4: 1495-1505 (1992) and Spencer *et al.*, *Plant Mol. Biol.* 24: 51-61 (1994).

Following transformation of maize target tissues, expression of the above-described selectable marker genes allows for preferential selection of transformed cells, tissues and/or plants, using regeneration and selection methods now well known in the art.

After transformation of a plant cell or plant, plant cells or plants transformed with the desired DNA sequences integrated into the genome can be selected by appropriate phenotypic markers. Phenotypic markers are known in the art and may be used in this invention.

Confirmation of transgenic plants will typically be based on an assay or assays or by simply measuring growth rate. Transformed plants can be screened by biochemical, molecular biological, and other assays. Various assays may be used to determine whether a particular plant, plant part, or a transformed cell shows an increase in enzyme activity. Typically, the change in expression or activity of a transformed plant will be compared to levels found in wild type (e.g., untransformed) plants of the same type. Preferably, the effect of the introduced construct on the level of expression or activity of the endogenous gene will be established from a comparison of sibling plants with and without the construct. Cyclin, CDC25, Nim1, and P1x1 transcript levels can be measured, for example, by Northern blotting, primer extension, quantitative or semi-quantitative PCR (polymerase chain reaction), and other methods well known in the art (See, e.g., Sambrook, *et al.* (1989). Molecular Cloning, A Laboratory Manual, second edition (Cold Spring Harbor Laboratory Press), Vols. 1-3). Protein can be measured in a number of ways including immunological methods (e.g., by Elisa or Western blotting). CDK activity can be measured in various assays as described in Sun *et al.*, Proc. Nat'l. Acad. Sci. U S A. 96(7):4180-85 (1999). Cell division of a plant cell or tissue can be measured in a variety of ways including those described in Myers *et al.*, Plant Physiol. 94:1330-36 (1990) and Artlip, *et al.*, Plant Cell and Environ 18:1034-40 (1995).

Normally, regeneration will be involved in obtaining a whole plant from a transformation process. The term "regeneration" as used herein, means growing a whole plant from a plant cell, a group of plant cells, a plant part, or a plant piece (e.g., from a protoplast, callus, or a tissue part).

The foregoing methods for transformation would typically be used for producing transgenic inbred lines. Transgenic inbred lines could then be crossed, with another (non-transformed or transformed) inbred line, in order to produce a transgenic hybrid maize plant. Alternatively, a genetic trait which has been engineered into a particular maize line using the foregoing transformation techniques could be moved into another line using traditional backcrossing techniques that are well known in the plant breeding arts. For example, a backcrossing approach could be used to move an engineered trait from a public, non-elite line into an elite line, or from a hybrid maize plant containing a foreign gene in its genome into a line or lines which do not contain that gene. As used herein, "crossing" can refer to a simple X by Y cross, or the process of backcrossing, depending on the context.

Various plants will be suitable targets for enhancing cell division in female reproductive organs with the identified genes. In particular, the methods of the invention described herein may be applicable to any crop species including but not limited to barley, sorghum, wheat, maize, soybean, and rice.

In a most preferred embodiment, transformation is carried out in maize plants according to the method of Agrobacterium.

Parts obtained from the regenerated plant, such as flowers, pods, seeds, leaves, branches, fruit, and the like, are covered by the invention, provided that these parts comprise cells which have been so transformed. Progeny and variants, and mutants of the regenerated plants are also included within the scope of this invention, provided that these parts comprise the introduced DNA sequences.

Cyclin, CDC25, Nim1,and P1x1 levels and the activity of CDK are preferably determined as set forth in the examples.

Once a transgenic plant is produced having a desired characteristic, it will be useful to propagate the plant and, in some cases, to cross to inbred lines to produce useful hybrids.

In seed propagated crops, mature transgenic plants may be self crossed to produce a homozygous inbred plant. The inbred plant produces seed containing the genes for the newly introduced trait. These seeds can be grown to produce

plants that will produce the selected phenotype. All articles cited herein and in the following list are hereby expressly incorporated in their entirety by reference.

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All references cited herein are hereby expressly incorporated in their entirety by reference.

What is claimed is:

1. A recombinant expression construct for production of plants that have enhanced yield potential comprising: a recombinant cell division enhancing nucleotide sequence, and
regulatory elements that will provide for expression of said sequence in a plant cell.

2. The expression construct of claim 1 further comprising a promoter operably linked to said sequence, said promoter being one which provides temporal and spatial expression during anthesis development.

3. The expression construct of claim 1 where said promoter provides expression during the exponential growth phase of the ear.

4. The expression construct of claim 1 wherein said promoter provides expression during the lag phase of development of the kernel.

5. The expression construct of claim 1 wherein said promoter provides expression from about 14 days prior to about 12 days after pollination.

6. An expression construct for production of transgenic plants that will enhance yield potential comprising: a cell division enhancing nucleotide sequence and a promoter operably linked to said sequence, said promoter being one which gives temporal and spatial expression of said construct during anthesis development, said cell division enhancing nucleotide sequence being one which encodes upon expression a protein that activates or modulates cyclin-dependent kinases in female reproductive organs.

7. The expression construct of claim 6 where said promoter provides expression during the exponential growth phase of the ear.

8. The expression construct of claim 6 wherein said promoter provides expression during the lag phase of development of the kernel.

9. The expression construct of claim 6 wherein said promoter provides expression from about 14 days prior to about 12 days after pollination.

10. The expression construct of claim 6 wherein said nucleotide sequence comprises: a DNA sequence encoding a gene product useful for affecting expression of a protein selected from the group consisting of B-type cyclins, D-type cyclins, CDC25, Nim1, P1x1, and Wee1 in a plant or plant tissue.

11. The expression construct of claim 6 wherein said nucleotide sequence includes natural variants of genes enhancing reproductive cell division.

12. The expression construct of claim 6 wherein said promoter is a maternal tissue promoter.

13. The expression construct of claim 6 wherein said promoter is selected from a group consisting of zag2, ltp2, gamma-zein, cim1, mze40-2, b22e, end1, and bet11.

14. The expression construct of claim 6 wherein said promoter is an inducible promoter.

15. An expression construct useful for the production of a transgenic plant with improved yield potential, the construct comprising: a recombinant gene or combination of genes which encode upon expression a protein which increases cell division in female reproductive organs; and a promoter operably linked to said gene or genes, said promoter being one which gives temporal and spatial expression of said gene products during anthesis.

16. The expression construct of claim 15 where said promoter provides expression during the exponential growth phase of the ear.

17. The expression construct of claim 15 wherein said promoter provides expression during the lag phase of development of the kernel.

18. The expression construct of claim 15 wherein said promoter provides expression from about 14 days prior to about 12 days after pollination.

19. The expression construct of claim 15 wherein said gene or combination of genes comprises: a DNA sequence encoding a gene product useful for affecting expression of a protein selected from the group consisting of B-type cyclins, D-type cyclins, CDC25, Nim1, P1x1, and Wee1 in a plant or plant tissue.

20. The expression construct of claim 15 wherein said genes include natural variants of genes enhancing cell division.

21. The expression construct of claim 15 wherein said gene construct includes a maternal tissue promoter.

22. The expression construct of claim 15 wherein said promoter is selected from a group consisting of zag2, ltp2, gamma-zein, cim1, mze40-2, b22e, end1, and bet11.

23. The expression construct of claim 15 wherein said promoter is an inducible promoter.

24. The expression construct of claim 15 wherein said plant is selected from the group consisting of maize, barley, sorghum, soybeans, wheat, rice, and *Arabidopsis*.

25. A transgenic plant comprising a plant cell or ancestor thereof which has been transformed with the expression construct of claim 1.

26. A method of increasing yield potential in a plant comprising: introducing to a plant cell a genetic construct, said genetic construct comprising a recombinant nucleotide sequence which encodes upon expression a protein which is associated with activating or modulating cyclin-dependent kinases in the female reproductive organ of said plant, and a promoter operably linked to said nucleotide sequence, said promoter being one which gives temporal and spatial expression of said sequence during anthesis development; and, said genetic construct is integrated into said plant cell.

27. The expression construct of claim 26 where said promoter provides expression during the exponential growth phase of the ear.

28. The expression construct of claim 26 wherein said promoter provides expression during the lag phase of development of the kernel.

29. The expression construct of claim 26 wherein said promoter provides expression from about 14 days prior to about 12 days after pollination.

30. The expression construct of claim 26 wherein said nucleotide sequence further comprises: a DNA sequence encoding a gene product useful for affecting expression of a protein selected from the group consisting of B-type cyclins, D-type cyclins, CDC25, Nim1, P1x1, and Wee1 in a plant or plant tissue.

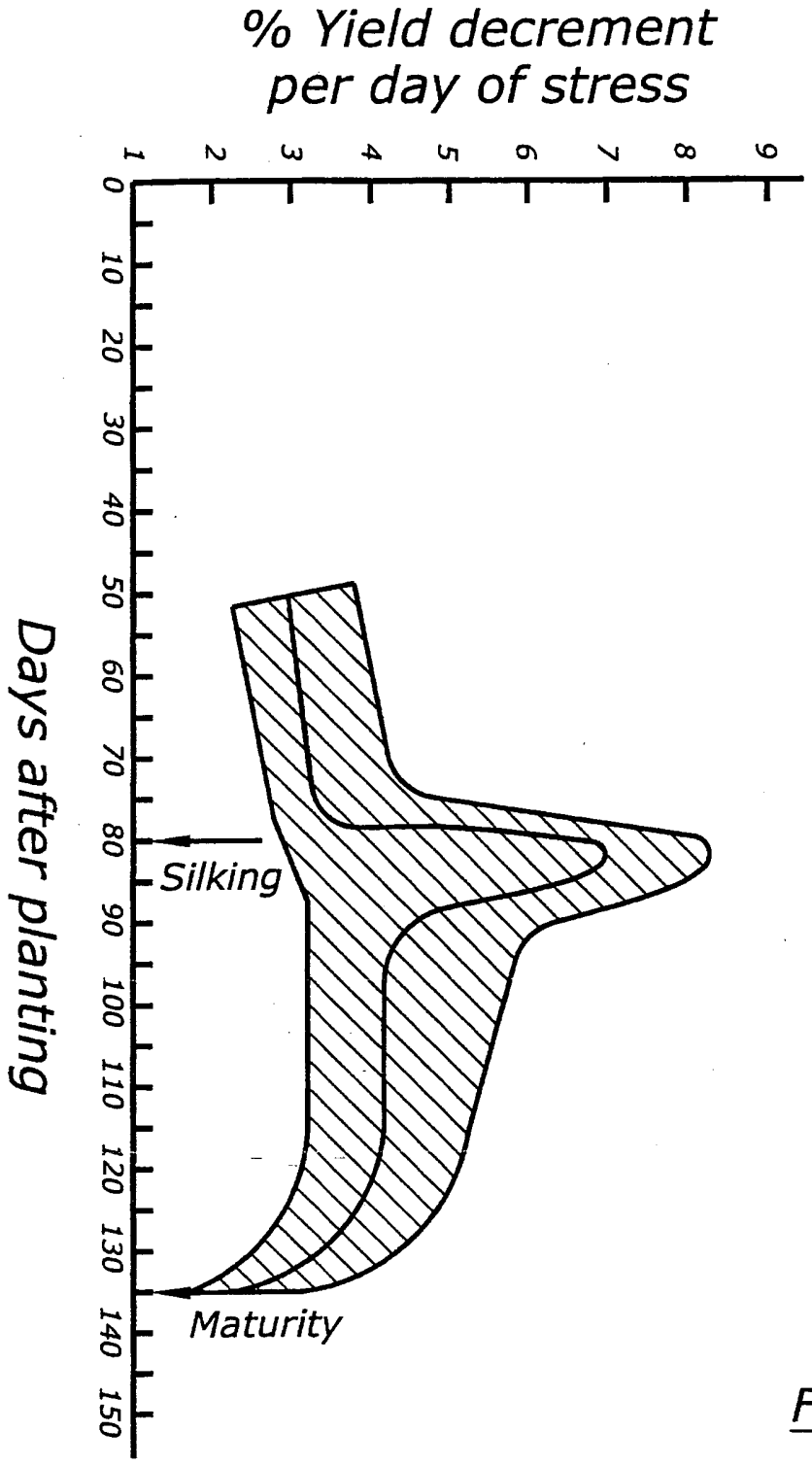
31. The method of claim 26 wherein said genes include natural variants of genes enhancing cell division.

32. The method of claim 26 wherein said gene construct includes a maternal tissue promoter.

33. The method of claim 26 wherein said promoter is selected from a group consisting of zag2, ltp2, gamma-zein, cim1, mze40-2, b22e, end1, and bet11.

34. The method of claim 26 wherein said promoter is an inducible promoter.

35. The method of claim 26 wherein said plant is selected from the group consisting of corn, barley, sorghum, soybeans, wheat, rice, and *Arabidopsis*.


% Yield decrement
per day of stress
1
2
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5
6
7
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10
20
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70
80
90
100
110
120
130
140
150
Days after planting
Silking
Maturity

Fig. 1

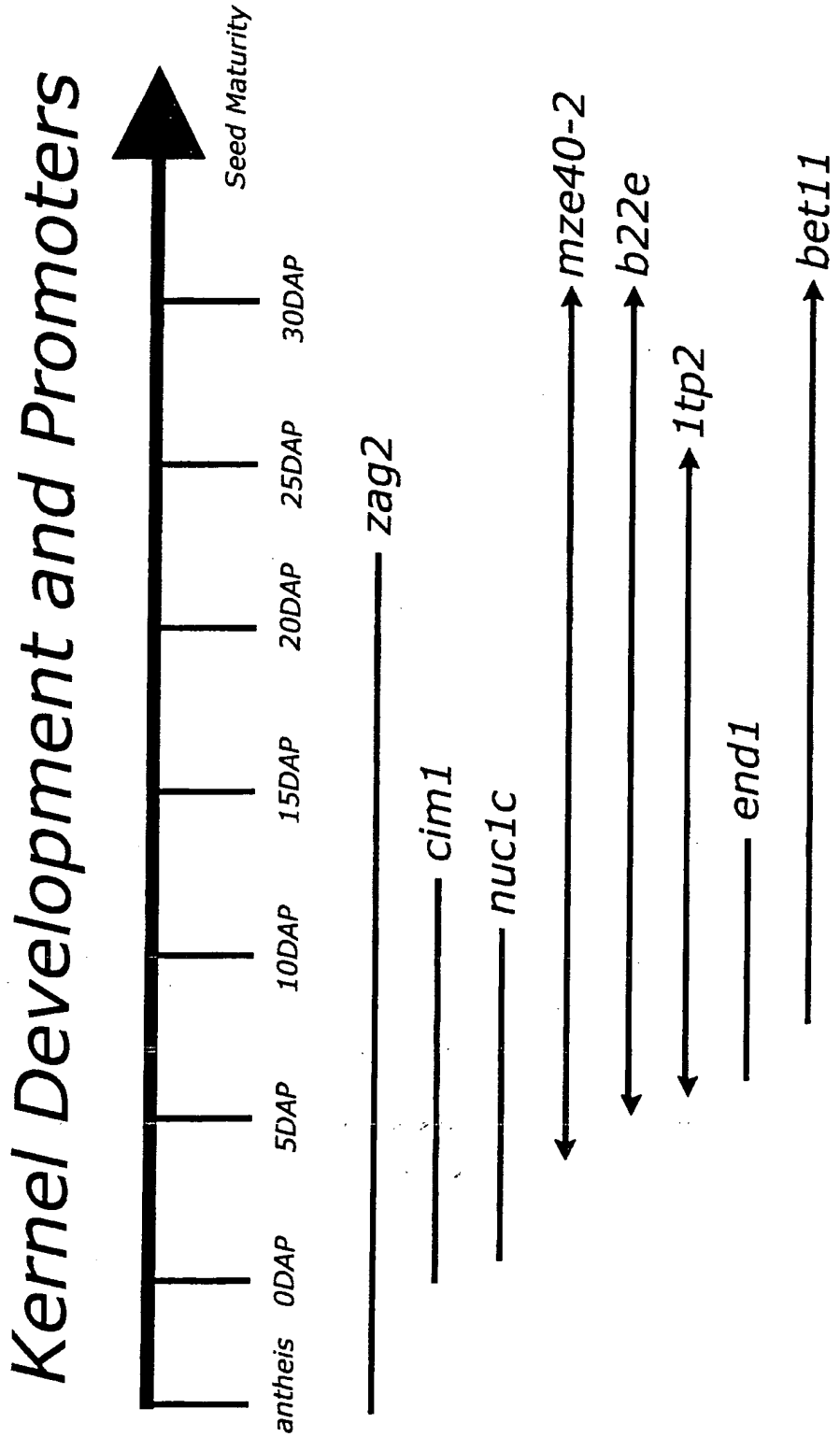
Kernel Development and Promoters
antheis
0DAP
5DAP
10DAP
15DAP
20DAP
25DAP
30DAP
Seed Maturity
zag2
cim1
nuc1c
mze40-2
b22e
1tp2
end1
bet11

Fig. 2